



## #398: The baby makers: The science behind healthier embryos and better IVF

### VOICEOVER

This is Up Close, the research talk show from the University of Melbourne, Australia.

### DR ANDI HORVATH

Hi I'm Andi Horvath, thanks for joining us. In the late 20th century scientists developed IVF to assist couples who couldn't conceive naturally. The term in vitro fertilisation, IVF, actually refers to the creation of an embryo in a dish, but that's really only half the picture. An embryo then has to be transferred to a uterus and implantation has to take place. In the early days of IVF, multiple embryos were transplanted increasing the chances that one would take, but often the result was multiple births, such as triplets, bringing with it a new set of pregnancy and parenting complications. But IVF has moved on with improvements to the technology.

Our guest today on Up Close is reproductive biologist, David Gardner. He's been instrumental in growing our understanding of what makes for a viable embryo and the optimal conditions needed for embryos to thrive after transplantation. David's work has contributed to making single embryo transfer as the standard for IVF today and as David will tell us, studying embryo viability is giving us unexpected insights into the world of cell growth and proliferation beyond IVF including potentially how cancers take hold.

Professor David Gardner is a member of the Australian Academy of Science and a professor in the School of Biosciences, University of Melbourne. He's the author of 15 books and countless articles on embryology and IVF and is well known as a guru of human embryo development. David, welcome.

### DAVID GARDNER

Thank you.

### DR ANDI HORVATH

When were researchers first able to fertilise an egg with sperm outside the

mammalian body and see this replicating ball of cells?

DAVID GARDNER

Well it was really efforts at Cambridge in the laboratory of Robert Edwards where they were able to get various eggs from different species and establish how to do in vitro fertilisation. But it was really only in the late 1970s that it was possible to get a routine source of human eggs, of human oocytes and that really was a serendipitous chance finding that came about when Professor Edwards was at a seminar with Patrick Steptoe, who's a gynaecologist. Patrick was able to show how to use a laparoscope to look inside a patient. When Bob saw this he immediately realised that this was a way to source human oocytes.

So this wonderful collaboration between a scientist and a physician that established means of collecting human eggs and ultimately the fertilisation of the human egg in a culture dish and this occurred in the 1970s. Finally, they were able to translate this into a clinical practice and the first fertilised egg was transferred successfully into a patient in 1978 in Oldham in the North of England. Nine months later, the miracle baby, Louise Brown, was born in July.

DR ANDI HORVATH

There's a potted history of IVF that we needed. Now tell us the facts of life. What happens immediately after conception?

DAVID GARDNER

The fertilised egg undergoes a series of divisions. So to understand what happens is that the egg is the largest cell in the female body. It's about 110 microns across. So it's just smaller than a full stop on a printed page, but it's a very big cell. So the first thing that happens after the sperm enters the cell is that the embryo goes through divisions. But the cells actually get smaller and these are called cleavage divisions and the embryo's called a cleavage stage. So we have one, two, four, eight cells and it's at this time of development that the embryo's normally transferred, back in the 70s and 80s, to the uterus of the patient.

DR ANDI HORVATH

When did they first realise that a ball of cells outside the dish could go back into the body?

DAVID GARDNER

Well it was known for decades that you could do this in animal models. So in mice and in sheep you could grow embryos and transfer them and that was during the 1960s and 1970s. So the proof of principle study was done many years before. So we knew it could be done. It's really a question of being able to access the human egg. So it's really this breakthrough that Patrick Steptoe, the gynaecologist, had we've been able to access eggs through laparoscopy that led the way to creating the embryos.

I think it was always a given that those embryos would be able to be put back and

they would give rise to pregnancies.

DR ANDI HORVATH

So what makes the embryo attach to the uterine wall and why doesn't the uterine wall reject it?

DAVID GARDNER

Okay, so after five days of development, the embryo creates what we call a blastocyst, it's a big ball of cells. So at this point it's created an epithelium and it started to differentiate into two cell types, the outer cells go on to form placenta. Inside of this ball of cells is a small group of cells, about 20 cells, called the inner cell mass. It's from those cells that we get the baby. So implantation is an interaction between the outer layer of cells of the embryo, of the blastocyst with the lining of the uterus which we call the endometrium. It's a very elegant dialogue between the baby to be and the mother to be. The baby to be has to say to the mother to be, look I'm here, please don't have a menstrual cycle, please don't shed the lining of your uterus and at the same time the lining of the uterus has to be prepared enough for it to say, well welcome basically. I'm ready to receive you with open arms.

DR ANDI HORVATH

Tell us about some of the challenges for IVF technology before it became mainstream?

DAVID GARDNER

I think one of the biggest things is that we knew so little about the human embryo. We didn't know what it needed to grow. All our early attempts to grow the embryo were done using what we call tissue culture media or very simple salt solutions. They were empirical, they weren't actually based on what the embryo needed. It was rather like we were force feeding these embryos what we thought they should have rather than listening to them and say what would you like to eat? I think that was the big difference.

So in the early days, embryos really didn't grow very well and they only managed to survive typically for one to two days in the laboratory. So for fertilisation up to implantation it takes about five days. Clearly, we weren't able to maintain the later stages of growth in the culture dish and that was the problem. So as a result of this it became normal to put back these early embryos on day two or day three into the uterus, but here's the catch, those embryos don't belong in the uterus at that stage. They're normally residing in the fallopian tube, the oviduct. So we're putting back these embryos too soon.

Now what we know again from extensive research on several different mammalian species, whether it's a sheep or a cow or a mouse, that if you put the embryo back too early into the uterus it doesn't develop very well. The reason is simple, the oviduct and the uterus create different environments. So we're putting the embryo back into somewhere that it doesn't belong with the wrong nutrients and so it doesn't do very well.

DR ANDI HORVATH

Now in the early days of IVF they had to do multiple embryo transfers, why did they do that?

DAVID GARDNER

Well because we're putting back embryos, what we call asynchronously, we were putting back embryos at day two and day three back into the uterus. That environment did not support the embryo growth. So consequently, implantation was compromised. Only maybe 10 percent of those embryos, 15 percent of those embryos gave rise to an implantation. Now the patient has been through an awful lot to get to that period of the embryo transfer. So to increase the potential of a pregnancy, rather than have just one embryo put back, if you put two or three or even four back, as was very common in the 80s and 90s, certainly in the USA, you actually did increase the chances of the patient getting pregnant.

Now four percent of all pregnancies in Australia are multiple gestations but in the early days of IVF 25 percent of the multiples came from IVF cycles. So it's a bit of a paradox if you think about it. Infertility, infertile patients actually contributed the most to multiple gestations in every developed country and that's because we were putting back too many embryos.

DR ANDI HORVATH

Now infertility, what's the causes of infertility?

DAVID GARDNER

There are so many causes of infertility. Infertility affects one in six couples. One in six couples need medical intervention to conceive a child. I think people are often very surprised when they hear it's that common. It's something that people don't like to talk about. I refer to it as the silent sickness in society. For women, it's easier to discuss but for men it's something that they don't want to go down to the pub and say, hey guys I can't have a child. It's a very difficult topic for them.

So in about a third of the cases of infertility, it's maybe a tubal factor for the woman, she has blocked tubes or there's some endometrial problems. So in other words, her uterus lining won't receive the embryo. There are many other causes, but it's about a third of the cases are attributed to the woman. A further third are attributed to the men in which he has problems producing sperm and the sperm are either too few or abnormal. Then we have the other third which we call idiopathic in which we just don't know. It could be both the patients are suffering subfertility but in those cases often the diagnosis is not that clear.

DR ANDI HORVATH

So how does the quality of the gametes, the egg and sperm matter when creating a viable embryo?

DAVID GARDNER

It is important to look after your eggs and to look after your sperm. It sounds quite

obvious. When you're a parent you do everything you can to look after your child, from birth all the way through to adolescence. You do absolutely everything. You go out of the way to optimise their nutrition, to look after their welfare and yet it's ironic that before birth people don't really consider the health of their gametes, the very gametes that are going to give rise to the child. Well it does transpire, it's terribly important to look after your sperm or your eggs.

One of the best things you can do is through diet. It's become evident now through research around the world, including our laboratory, in this case in animal models, if either the male or the female becomes obese through inappropriate diet then the sperm and the eggs in due course become compromised. You can see this quite clearly, you see lower fertilisation rates, but the embryos don't develop as well. This, we now know is then translated further down into fetal development and into adulthood. So it's really important that we think about getting fit for conception. That we actually consider the health of our gametes when we're thinking of the health of our future children.

DR ANDI HORVATH

I'm Andi Horvath. We're talking about how in vitro embryo technologies developed, and where they're going with embryologist, David Gardner, here on Up Close. Now explain for us a little bit about epigenetics. The role of say even the womb environment on the growing embryo. Now there's some famous historic research I know you're going to share with us.

DAVID GARDNER

Right, the term epigenetics means above the gene really. So it's looking at how your genes are expressed without changes to the actual structure of the genes themselves. So things that actually regulate the turning on and turning off of your genes and a lot of those can be environmental and a lot of those are diet.

So in the Second World War, there was a very famous case of a Dutch famine where the Germans blockaded food to the Dutch people and caloric restriction, their calorie intake was about a third of what it should have been. A lot of those women were actually pregnant at the time. So they actually went through a whole pregnancy or part of a pregnancy with insufficient calories. It transpired when those children were then followed on into adulthood they had an increased risk for cancers, heart disease and other morbidities. These were translated all the way back to development in utero.

Since then a theory called the Developmental Origins of Health and Disease has evolved in which it's implicated that events during your life in utero, in other words what your mother eats, what she's exposed to whether it's smoking or whatever, pollutants, will affect not only your growth as a fetus but will affect your subsequent health and ultimately how you will die as an adult. What we're learning now is that that goes all the way back to the embryo before implantation and it goes back to the gametes before fertilisation.

So really this whole concept of being fit for fertility is about your gametes, it's about your embryo, it's about your fetus and then, of course, once we have our lovely children, it's about taking them all the way to adulthood, but it starts as a single cell.

DR ANDI HORVATH

David, explain the stresses on a ball of embryonic cells outside the womb because that's not in its normal environment. So we're creating a different environment when we're nurturing it and that must have an effect, or does it?

DAVID GARDNER

No, it does. When we grow an embryo in a culture dish, we have to be very mindful that we maintain things like temperature at all times because that can affect the physiology, the pH, that's the levels of acidity and alkalinity have to be very tightly controlled, just as they are in the human body. So we have to use special incubators that can regulate, the precise pH for the embryos to develop.

With regards to the environment, the culture environment, as I alluded to in the early days of IVF those solutions were pretty much empirically developed. The turning point from our perspective was really, in my early days of human IVF working with Carl Wood and John Leeton, two of the pioneers here in Australia, and I said to them, well I would love to know what's inside the human oviduct and the human uterus so I could recreate a better culture environment for the embryos.

Both John and Carl were able to do that. They got me samples of oviduct and uterine fluids and our team analysed them for nutrients. It was from there that we created two media, one based around the oviduct and one based around the uterus. From that we were pleasantly surprised that we could actually maintain the human embryo to the blastocyst stage. So we could grow a one cell embryo for five days to 100 cells, readily in vitro. That had not been done routinely before. That was the turning point because once we'd developed these blastocysts, we were able to put them back into the uterus at a time they would normally enter. The consequence of which was the implantation rates doubled.

DR ANDI HORVATH

So that meant you no longer had to do multiple embryos as well.

DAVID GARDNER

Yeah, that's exactly right. One of the big problems, certainly in America was this high order multiple gestations. So in the mid-90s, the average number of embryos being transferred in the USA in an in vitro fertilisation clinic was five. So that's a lot. Consequently, there were lots of twins and there were lots of triplets. There was a population that desperately needed to reduce the number of embryos being transferred. So in the mid-90s I moved to America to implement clinically this blastocyst transfer technology. We were able to show conclusively in prospective randomised trials that implantation rates were significantly higher per embryo. Meaning that we could get patients pregnant with significantly fewer embryos. Ultimately as we improved the technology we got down to just transferring one

embryo at a time.

DR ANDI HORVATH

Tell me more about the embryo cell culture, I'm curious. You mentioned temperature, pH, did you use antioxidants, what's involved in creating an environment for a viable embryo?

DAVID GARDNER

So one of the things we learnt from analysing oviduct uterine fluid was that there were gradients of nutrients and these nutrients were very striking at first glance. So the oviduct doesn't have much glucose in it, but it does have a lot of what we call pyruvic acid and lactate. Conversely the uterus has a lot of glucose but very low levels of the pyruvate and lactate. It mirrored what we already knew about what the embryo needed. When we asked the embryo, what did you need and we were able to answer that question by very clever micro-analytical technologies we developed.

The embryo says that pretty much early on I don't want glucose. I need these other acids and I need some amino acids. Later on, as the embryo develops and becomes more complicated it needs a lot of glucose and it needs a wider variety of amino acids to fulfil several niches in their physiologies. So it was really understanding the complexities of how the embryo changed. When I first started studying embryo physiology I couldn't decide whether I should be a physiologist or a psychiatrist because every day the tissue became something different. It was changing its personality and that was gorgeous because every day it was taking on more and more of an adult phenotype as it progressed.

DR ANDI HORVATH

Wow, so you were monitoring its glucose needs and its protein needs and you were also noticing the various acids that it was making from glucose metabolism, like lactate?

DAVID GARDNER

Right.

DR ANDI HORVATH

So David is there an algorithm that you can make to decide what is a viable embryo by looking at it, by testing nutrients, that sort of thing?

DAVID GARDNER

That's kind of the holy grail of IVF. How do we pick the embryo? We create on average 10 embryos. How do we know which one to transfer? Of those 10 we create, five or six will form blastocysts, but which one do we put back. So there are various things you can do. Over recent years we've had access to time lapse technology and through time lapse we're able to look at the embryo in real time every 10 minutes. Then we get a really interesting history of how these embryos develop. So using this technology which has been referred to as morphokinetics, so morphology and time kinetics, morphokinetics, we and others have put together

various attempts at creating algorithms, mathematical models that will help us select the best embryo. The data's really quite promising with those, but prospective trials have yet to be fully undertaken.

The other thing you can do though is you can measure what the embryo consumes. We've done this in animal models and what we've shown conclusively and in a pilot, clinical study was that the embryo, certainly at the blastocyst stage consumed, in this case, the most glucose which is their preferred nutrient, have a much higher pregnancy potential. So currently our research is looking at taking these algorithms where we've looked at the embryo and at the same time we're starting to measure what each embryo was doing and so we're creating a biochemical fingerprint as well. Therefore, we're having all this data helping us now to select those embryos for transfers.

DR ANDI HORVATH

On Up Close today we're talking about the new insights coming out of research into embryo viability with reproductive biologist, David Gardner. I'm Andi Horvath.

I want to come back to the nutrients the embryo uses and the metabolites that you measure. So lactate which is made from using glucose in the cells is instrumental for the uterine wall. Explain that to us.

DAVID GARDNER

Well this is recent work and ongoing. So it's really more of a creative hypothesis than fact. What we know, and what we've known for many years, is that the embryo has a really idiosyncratic metabolism at the blastocyst stage. So just before implantation this beautiful ball of cells does something very, very strange. It consumes a lot of glucose and it can oxidise it but it chooses also to put into the environment a lot of lactic acid. Now most tissues do one or the other, you either oxidise all the glucose, or if you're an athlete or when you train and you run you become anaerobic and you produce a lot of lactate and you don't oxidise that glucose.

DR ANDI HORVATH

Right, that's that pain in your muscles, isn't it?

DAVID GARDNER

Exactly, that's when you have to stop, that's the cramp. So it puzzled us, why does the embryo do that? When I went to the literature to find out what other cells behave this way, I really could only find one and that was a cancer. Indeed, this phenomenon of being able to produce lots of this lactate that normally you get after running, but producing it all the time, was observed in cancers in the 1920s. A German biochemist by the name of Otto Warburg who won the Nobel Prize for this observation determined that cancers produced lots of lactate. But he thought that they did it because there was a miss-function of their mitochondria which are the organelles that your cells use to oxidise things. But it turns out that wasn't true.



The cancer has mitochondria that can oxidise, they just choose to produce a lot of lactate. Same with the blastocyst. So for many years I said, well that's interesting embryos are like cancers. A few years ago, I had what I would call a bit of a light bulb moment when I thought, actually that might not be the case. Why do cells produce lots of lactate?

Well if you think about this embryo, this beautiful ball of cells it has to attach to the lining of the uterus. It then, like a cancer, has to invade. It has to basically disaggregate the cells of the mum and push itself inside. It's a very coarse term, but that's exactly what happens. Then it has to do two more things. It has to say, you know what, I need some blood. I need to establish a blood supply. We call it angiogenesis. So how does an embryo, how does a cancer trick - the embryo tricks the mum, but how does the cancer trick the host to give it blood. Then finally, of course, the embryo is technically a foreign tissue to the mother and a cancer is certainly a foreign tissue to us, how do you evade immune rejection? How do you stop the body killing us?

So these are the commonalities, these are the things that an embryo and a cancer have in common. They both need to invade. They both need to establish a blood supply and they both have to say don't kill me. So through extensive research and reading, it turns out that lactate can act as a signal to tissues to enhance the activities of enzymes that are involved in the breakdown of tissues. It's a signal to induce blood supply formation and finally it turns out it's an immunosuppressant.

So, if you can imagine, this embryo creating what I like to think of as this lactate cloud, it surrounds itself with this cloud of lactate which is also acidic. So it's an acid cloud and it's this acid cloud that facilitates tissue degradation, angiogenesis the formation of blood and the prevention of immuno-rejection. If you look what cancers do, that's exactly what cancers do. So whereas I used to think that embryos were like cancers, I don't think that anymore. I think that cancers are like embryos and that cancers use every trick in the book that the embryo has established to create a successful implantation.

DR ANDI HORVATH

So this gives us some clue to cell activity, cancers, what then next? Where do you see this research leading to?

DAVID GARDNER

Establishing a normal implantation is essential for the health of the baby. It's absolutely imperative that that dialogue between the embryo and the mum takes place. The problem, as you can imagine if you want to research that, is that you've got a uterus inside the body and then you've got an embryo. How do you study that interaction? It's very, very difficult. One of the things you could do is you can establish in vitro cells from the uterus and look how they interact with embryos. What we've been doing is been working with Frank Caruso here at the University of Melbourne who's a biomolecular engineer. With Frank, we've created what we call synthetic blastocysts and these are the same size as an actual human embryo, but

they're made out of polymers that we've created. They are able to release, in this case, lactate but they're also able to release other signals to the endometrial tissue and we can look for what kind of response they elicit in the uterus. That is helping us try to break down this dialogue. What are the signals that are essential for a normal implantation?

DR ANDI HORVATH

What about the converse, can we use this for cancer research?

DAVID GARDNER

Well the thing about implantation research or looking at these blastocysts, number one is that if you can understand what promotes implantation, technically you could also create a contraceptive. You can do the converse. That's somewhere where that research will definitely go in contraceptive research. The other thing is, yes, if you can work out what it is that establishes a dialogue between one invasive tissue to make another one receptive, it's plausible that lessons learnt from the blastocyst could be passed onto cancer research. Now to this point it's known that, for example, that you could reduce the availability of glucose to a cancer, can actually have a detrimental effect on that cancer's growth. So something as fundamental as blocking glucose utilisation could have a profound effect.

If you want to look holistically, I think this is when it gets really exciting is when we start talking about our diets. I mean we were talking about, further on, looking at our epigenetics and how we interact. I think this is now, this whole new era and if I may introduce you to a new -omic. We talk about epigenomics and genomics and proteomics.

DR ANDI HORVATH

Proteomics.

DAVID GARDNER

Exactly. Now we have metabolomics. One of the most exciting areas of our research in many of the laboratories around the world now is the meshing of metabolism and epigenetics. This is called the metaboloepigenome. Basically, what it means is that you are what you eat. That's the nicest way of looking at it. In terms of the embryos themselves, you are what your mum eats or your dad ate before you were conceived and we're learning fascinating things in this arena. I think there's some really important life lessons for all of us and it's all coming from our studies on these embryos before implantation.

DR ANDI HORVATH

So what's the next imperative for IVF? It's actually, can I guess, watch what you eat for breakfast, lunch and dinner.

DAVID GARDNER

I would love to see the way people think about having a family is that they need to get the message that before they come to a clinic that they need to get fit. It's fit for

fertility as it were, fit for reproduction and look after themselves because then you're going to have healthier gametes which in turn you may not need IVF. But for those who do need IVF, and there still would be many, because diet isn't going to overcome a blocked oviduct, a fallopian tube. It's not going to overcome a low sperm count per se, so you're still going to need some kind of intervention. But what we can say is that at least epigenetically and in terms of health, we're giving those gametes the best possible chance.

DR ANDI HORVATH

I'm going to ask a futuristic question, but why bother putting the fertilised embryo back into the uterus? Can't we just nurture it outside?

DAVID GARDNER

Sounds like a great idea and I'm sure there'll be many, many women listening to this would go, please do that, that would be great. However, our understanding of the embryo post implantation and again coming back it's so hard to study those embryos and their interactions with the mother, that right now I really think that is still science fiction but it's something that obviously we'll be working on.

DR ANDI HORVATH

Can we speculate further, what needs to happen?

DAVID GARDNER

That's a very good question. Fully understanding the process of implantation and what are the signals from the mum? What are the, what we call growth factors, the cytokines, what are these very complex signalling pathways that are elicited and how does the embryo respond to that. If you get those wrong you're going to create abnormal development. So it's really imperative that research continues to understand what it takes for a healthy embryo to interact and establish that first phase of pregnancy which is implantation. Until we understand that in its fullness I don't see how in the near future at least, we're able to successfully propagate those cells and by giving them all the signals that they need, because they're at the beginning of this growth trajectory and if we get it wrong early on, it'll put them on the wrong path for life.

So I think there's a lot of work needs to be done.

DR ANDI HORVATH

A lot of metabolomics.

DAVID GARDNER

A lot of metabolomics, a lot of proteomics, a lot of epigenomics.

DR ANDI HORVATH

David, thank you.

DAVID GARDNER

Thank you.

#### DR ANDI HORVATH

We've been speaking about embryo development and reproductive technologies and the new insights coming out of the research in those areas with embryologist Professor David Gardner. David is a member of the Australian Academy of Science and does his research at the University of Melbourne. You'll find a full transcript and more info on this and all our episodes on the Up Close website. By the way, if you like Up Close you may want to check out another one of our podcasts, Eavesdrop on Experts, which features stories of inspiration and insight in conversation with researchers.

Up Close is a production of the University of Melbourne, Australia. This episode was recorded on 22 June 2017. Producer was Eric van Bommel. Audio Engineering by Gavin Nebauer. I'm Dr Andi Horvath, cheers.

#### VOICEOVER

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